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Sugars and Desiccation Tolerance in Seeds[1]

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ABSTRACT

Soluble sugars have been shown to protect liposomes and lobster microsomes from desiccation damage, and a protective role has been proposed for them in several anhydrous systems. We have studied the relationship between soluble sugar content and the loss of desiccation tolerance in the axes of germinating soybean (*Glycine max* L. Merr. cv Williams), pea (*Pisum sativum* L. cv Alaska), and corn (*Zea mays* L. cv Merit) axes. The loss of desiccation tolerance during imbibition was monitored by following the ability of seeds to germinate after desiccation following various periods of preimbibition and by following the rates of electrolyte leakage from dried, then rehydrated axes. Finally, we analyzed the soluble sugar contents of the axes throughout the transition from desiccation tolerance to intolerance. These analyses show that sucrose and larger oligosaccharides were consistently present during the tolerant stage, and that desiccation tolerance disappeared as the oligosaccharides were lost. The results support the idea that sucrose may serve as the principal agent of desiccation tolerance in these seeds, with the larger oligosaccharides serving to keep the sucrose from crystallizing.

Most angiosperm seeds can survive desiccation but only at a discrete developmental stage. If they are dried before reaching the desiccation tolerant stage of maturity, they will not germinate (2, 26). Similarly, if they are dried after germination has progressed too far, they will not continue to germinate upon rehydration (4, 18, 26). The emergence of the radicle from the seed coat is generally considered to be the stage at which desiccation tolerance is lost during germination (26).

Water is important to organisms not only as a solvent for biochemical reactions, but as a stabilizer of structure. Hydrophilic and hydrophobic interactions impart structure to macromolecules and organelles within cells. Membrane structure, in particular, depends on these complex interactions, and is often regarded as a primary site of desiccation damage (6, 26). The water replacement hypothesis suggests that polyhydroxy compounds can substitute for water in stabilizing membrane structure in the dry state (7, 23, 30). In the case of membranes, the hydroxyl groups can hydrogen-bind to polar head groups (8), providing the hydrophilic interactions necessary for membrane structure and stability.

Evidence for this hypothesis has been gathered by Crowe and co-workers, who have demonstrated that the glucose-dimer, trehalose, can effectively replace water in artificial membrane systems (8, 9). Trehalose occurs in many desiccation tolerant organisms, but has not been reported to occur in angiosperm seeds. Other soluble sugars, especially sucrose, can assume a protective role in artificial membrane systems (10, 11). Sucrose is commonly found in seed embryos (3); therefore, the occurrence of soluble sugars in three types of germinating seeds was analyzed to search for any correlations between sugar content and the loss of desiccation tolerance.

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MATERIALS AND METHODS

Plant Material. Seeds of three species were used. Peas (*Pisum sativum* L. cv Alaska) were purchased from the Burpee Seed Company (Warminster, PA) in 1983; corn (*Zea mays* L. cv Merit) was purchased from the Asgrow Seed Company (Kalamazoo, MI) in 1987; and soybeans (*Glycine max* L. Merr. cv Williams) were grown and kindly donated by Professor Madison Wright in 1986. All seeds were stored at 4°C until use. Seeds were imbibed in wet paper rolls (Anchor Paper Company, St. Paul, MN) in the dark at 25°C for the desired periods.

Leakage of Electrolytes. Seeds were imbibed for periods up to and beyond radicle emergence, then transferred to a chamber containing saturated LiCl, which equilibrates to 11% RH (21). Here the seeds dried to approximately 8% moisture content (dry weight basis). This low moisture content is lethal to desiccation-intolerant tissues. After drying, the seeds were transferred to 100% relative humidity for 24 h for slow rehydration in order to minimize damage to cells from hydrational forces (15). Thus, leakage from cells damaged by desiccation should be the main source of the electrolytes measured. Groups of 10 axes were isolated and submerged in 15 mL of deionized water. Conductivity was monitored continuously with an ElectroMark Conductivity Meter (Markson Science, Inc., Del Mar, CA). The rate of leakage was measured after 15 min, by which time it was approximately constant. Dry weights were obtained from duplicate samples dried at 95°C for 5 d.

Emergence of Seedlings. Seeds were imbibed and dried as above, then planted in Cornellmix (a peat moss and vermiculite based mix) in fiber pots and placed in a greenhouse (12 h photoperiod, 25°C day/20°C night). Emergence from the soil and radicle growth were scored after 7 d.

Analysis of Soluble Sugars. Axes from imbibed seeds were isolated and lyophilized to dryness. Soluble sugars were extracted by homogenizing 50 mg of dry axes in distilled water with an internal standard (melezitose) present. The homogenate was centrifuged, the pellet washed with distilled water, and recentrifuged. The supernatants were combined and run through a mixed bed ion-exchange column (Dowex 50W and Amberlite IRA-145, acetate form) to remove charged contaminants before lyophilization. Dried extracts were dissolved in a mixture of 75% acetonitrile and 25% water, filtered through a 45 micrometer pore-size nylon filter (Gelman Sciences, Ann Arbor, MI), and analyzed by HPLC.

Sugars were separated on a Brownlee Amino Column (Santa Clara, CA), using a solvent gradient from 75% acetonitrile, 25% water to 60% acetonitrile, 40% water. Detection was by refractive index. A pumping system and detector from Waters (Milford, MA) were employed. Quantitation was achieved by comparing

peak areas of interest to the peak area of the internal standard. Standard curves, generated using sugars purchased from Sigma Chemical Company (St. Louis, MO), were used to obtain milligram values from the peak area ratios measured.

RESULTS

In an effort to determine at what time during imbibition tolerance of desiccation is lost, we monitored the germination (radicle growth) and shoot emergence of seeds that had been desiccated during imbibition. Figure 1 (bottom) shows the emergence of soybeans that were preimbibed, desiccated, and then planted. After 18 h of preimbibition, desiccation prevented the primary radicle from growing upon subsequent planting. Many seeds were able to form lateral roots and achieve shoot emergence from the soil in spite of the death of the primary radicle. This capability did not persist after 24 h of preimbibition. Similarly, most pea radicles did not survive periods of preimbibition longer than 18 h (Fig. 2, bottom). In corn, radicles did not emerge after desiccation following 36 h of preimbibition (Fig. 3, bottom). These times for the loss of desiccation tolerance coincide with the times of radicle emergence from the seed coat for each species—18 h for soybean, 24 h for pea, and 36 h for corn (data not shown).

The extent of membrane damage by desiccation at various stages of imbibition was estimated by measuring electrolyte leakage rates of imbibed, dried, then rehydrated axes. The rate of electrolyte leakage was low and constant when tolerant radicles were rehydrated; however, after tolerance was lost, the rate of rehydrational leakage increased two- to threefold (Figs. 1, 2, and 3, top). High leakage rates indicate that diffusion barriers had been disrupted, presumably by desiccation and/or rehydration

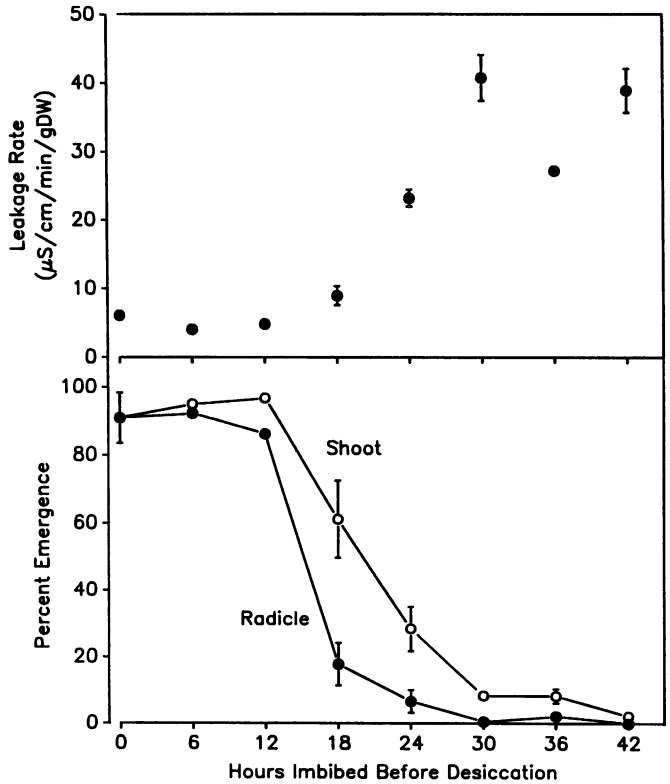

FIG. 1. Loss of desiccation tolerance during imbibition in soybean. Top, Rate of leakage from axes after imbibition, desiccation, and rehydration; bottom, emergence of radicles and shoots after desiccation following various lengths of preimbibition. Bars represent SE (n = 4, top; n = 3, bottom).

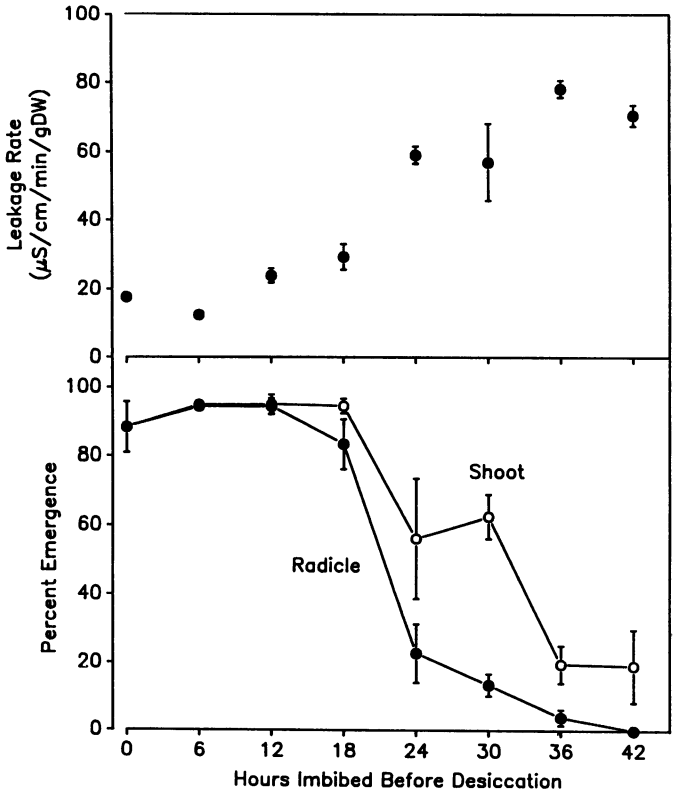

FIG. 2. Loss of desiccation tolerance during imbibition in pea. Top, Rate of leakage from axes after imbibition, desiccation, and rehydration; bottom, emergence of seed radicles and shoots after desiccation following various lengths of preimbibition. Bars represent SE (n = 4, top; n = 3, bottom).

(26, 27). The increased rates of leakage suggest that, in soybean and pea, membrane damage due to desiccation increases markedly between 18 and 24 h of imbibition (Figs. 1 and 2, top). In corn, leakage rates rise dramatically following desiccation after 24 h of imbibition (Fig. 3, top).

Soluble sugar contents in the imbibing axes were analyzed to discover any correlation between the loss of desiccation tolerance and sugar content. The data for sugar contents of radicles during germination (Figs. 4–6) show that sucrose and sucrosyl-oligosaccharides, particularly raffinose and stachyose, were abundant in the axes in early stages of germination. At about the stage where desiccation tolerance was lost, oligosaccharide content dropped sharply, although sucrose was still present. As oligosaccharides declined, monosaccharides rose, becoming the predominant soluble sugars present after the loss of desiccation tolerance. Monosaccharides were not individually identified by this HPLC technique; however, the retention time of the major monosaccharide peak corresponded to that of glucose.

DISCUSSION

Both the emergence and leakage studies confirm that desiccation tolerance of the radicle is lost soon after the radicle emerges from the seed coat. This is not to say that the entire seed is made nonviable by desiccation at this time; on the contrary, many seeds form lateral roots when the primary radicle is killed, and are still able to germinate. This phenomenon was also observed by Nemmer and Luyet (18), who reported that in pea, the radicle tip was the first tissue killed by desiccation after imbibition. These authors also frequently noted the appearance of lateral roots after the primary radicle had been killed by desiccation. Imbibition for slightly longer periods does cause the loss of

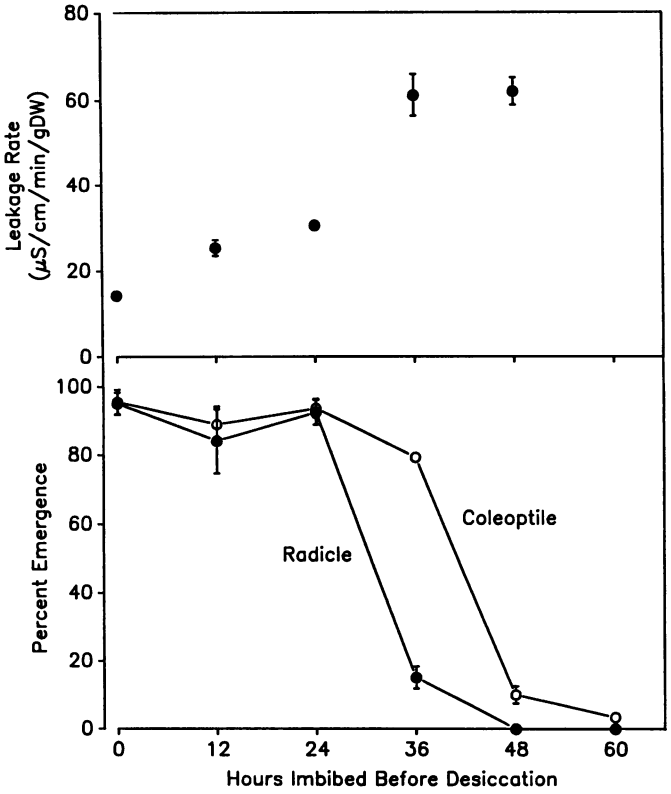

FIG. 3. Loss of desiccation tolerance during imbibition in corn. Top, Rate of leakage from axes after imbibition, desiccation, and rehydration; bottom, emergence of seed radicles and coleoptiles after desiccation following various lengths of preimbibition. Bars represent SE (n = 4, top; n = 3, bottom).

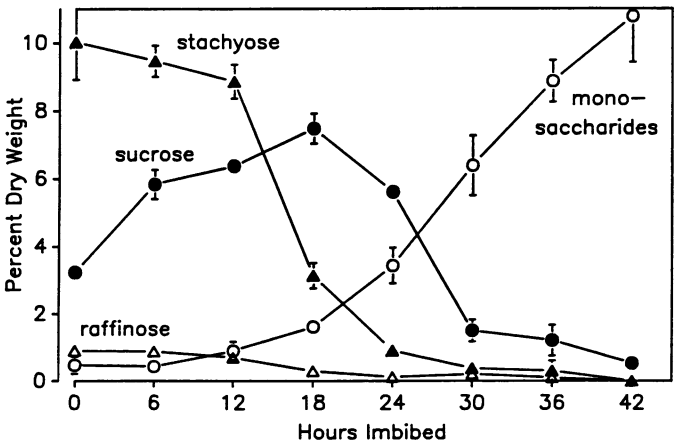

FIG. 4. Changes in soluble sugars of soybean axes during imbibition. Bars represent SE (n = 3).

desiccation tolerance in the entire seed, after which no shoot emergence occurs (Figs. 1–3, bottom).

As imbibition progresses, oligosaccharide content in these seeds decreases (Figs. 4–6). In each case, the loss of desiccation tolerance is coincident with the loss of nonsucrose oligosaccharides. Sucrose content may still be as high as 5 to 10% in the intolerant radicles, though it is generally decreasing at this time. Thus, the presence of sucrose without the larger oligosaccharides does not seem to confer desiccation tolerance. Several workers have reported oligosaccharide losses during imbibition in soybean (1, 12, 14). In these reports, the content of stachyose decreased before that of sucrose, while monosaccharide contents

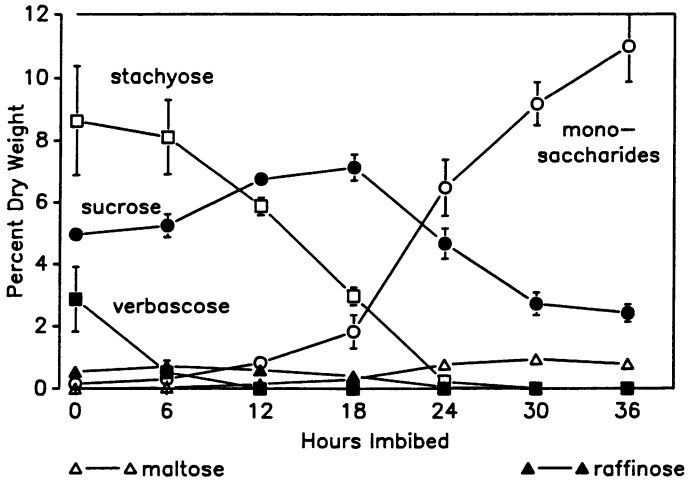

FIG. 5. Changes in soluble sugars of pea axes during imbibition. Bars represent SE (n = 3).

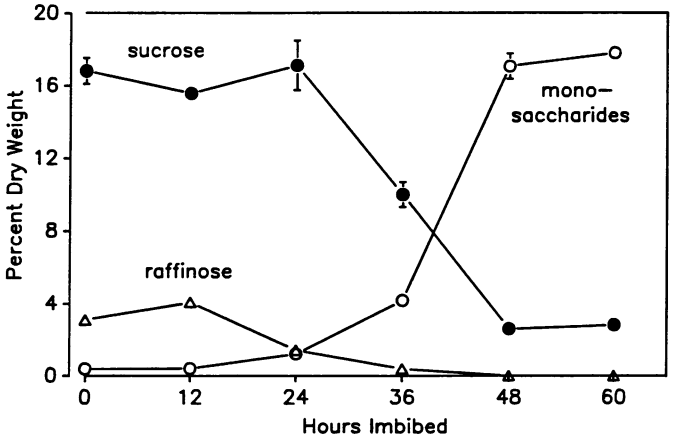

FIG. 6. Changes in soluble sugars of corn axes during imbibition. Bars represent SE (n = 3).

increased. The disappearance of oligosaccharides larger than sucrose at the time when desiccation tolerance is lost supports the idea that oligosaccharides may be necessary for desiccation tolerance in seeds, as suggested by Leopold and Vertucci (15).

Sucrose is an effective membrane protectant in model systems (10, 11, 17). The hydroxyl groups of sucrose may replace water by hydrogen-binding to the phospholipid head groups of the membrane (8, 29). If sucrose crystallizes, as is favored by slow drying (16), these hydroxyls will be unavailable. Alternatively, sucrose can form an amorphous glass during drying. In this state, the hydroxyls of the sugars would be free to bind to the membrane. Evidence for glass formation in corn embryos has been detected by Williams and Leopold (31). Glass formation may be favored by the presence of oligosaccharides, which disrupt normal crystal matrices (5, 28). Smythe (28) reported that among many sugars and other organic compounds tested, raffinose and stachyose were the most effective inhibitors of sucrose crystal growth. He also demonstrated that small amounts of these oligosaccharides suffice to suppress the sucrose crystal growth rate.

Evidence for the interaction of sucrose with phospholipids has been provided by Caffrey *et al.* (5), who report that sucrose can lower the chain order/disorder transition temperature of a dry phospholipid to that of a wet phospholipid. They also show that an oligosaccharide, raffinose, can effectively prevent the crystallization of sucrose.

Sucrose has been shown to protect isolated thylakoids from desiccation damage *in vitro* (25). Freeze-dried spinach thylakoids

retained approximately 50% of their photophosphorylation activity when dried in the presence of sucrose. Glucose and fructose were also effective, but sorbitol and proline were not. Schwab and Heber (25) have shown that the resurrection plants *Craterostigma plantagineum* and *Ceterach officinarum* contain about eight times the amount of soluble sugar as desiccation-intolerant spinach does. In a related paper, Schwab and Gaff (24) demonstrated that two species of desiccation tolerant grass accumulate large amounts of sugar (from 20–35% on a dry weight basis) as they dry. A related grass species that is not desiccation tolerant did not accumulate substantial amounts of sugar during drying. Unfortunately, the sugars were not identified, but were reported only as total weight of sugar per unit of leaf dry weight.

Ovcharov and Koshelev (20) have reported the loss of raffinose and sucrose during storage of corn seeds. After prolonged storage at three different relative humidities, the germinability of corn was 96, 12, and 0%. Corn with a final germinability of 96% contained both sucrose and raffinose. Corn with a final germinability of 12% only contained sucrose, and nongerminable corn contained neither sucrose nor raffinose. In the nongerminable corn, glucose and fructose had appeared.

A feature in common among the three species studied here is that monosaccharides, for the most part glucose, are predominant in the axes after desiccation-tolerance is lost (Figs. 4–6). Glucose, a reducing sugar, can participate in the Maillard reaction, a complex series of nonenzymic reactions that can lead to hundreds of end products, depending upon the reaction conditions (19). The reaction occurs with an amino group that is usually, but not always, on a protein. The Maillard reaction has been reported to cause protein inactivation and DNA damage (13). The first steps of the reaction can occur at low moisture contents (22), such as those experienced by drying seeds. Possibly, the Maillard reaction may contribute to the damage to desiccation-intolerant axes.

The analyses reported here indicate that the changes in soluble sugar contents in the axes of three seed species can be correlated to the loss of desiccation tolerance. First, the loss of desiccation tolerance corresponds to the loss of oligosaccharides, which may serve to prevent sucrose crystallization. In the noncrystalline form, sucrose may interact with membrane surfaces, possibly replacing water in the maintainance of membrane structure. Also, the loss of desiccation tolerance coincides with an increase in reducing-monosaccharide content of the axes. The accumulation of reducing sugars in a drying seed could lead to the occurrence of the Maillard reaction, which may cause protein and nucleic acid damage, thus threatening the viability of the seed.

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